

DreamTaq

	50 μ L	20 μ L	Step	Temp, $^{\circ}$ C	Time	Cycles
Water	37.75-X	15.4-X	Initial denaturation	95	1-3 min	1
10X DreamTaq Buffer	5	2	Denaturation	95	30 s	25-40
dNTP Mix (10 mM each)	1	0.5	Annealing	Tm-5	30 s	
Forward primer (10 μ M)	3	1	Extension	72	1 min/kb	
Reverse primer (10 μ M)	3	1	Final Extension	72	5-15 min	1
DreamTaq (5 U/ μ L)	0.25	0.1				
Template DNA	X (0.01-1000 ng)	X (0.004-400 ng)				

Phusion High-Fidelity DNA Polymerase

	50 μ L	20 μ L	Step	Temp, $^{\circ}$ C	Time	Cycles
Water	32.5-X	13.3-X	Initial denaturation	98	30 s-3 min	1
5X Phusion HF Buffer	10	4	Denaturation	98	5-10 s	25-40
dNTP Mix (10 mM each)	1	0.5	Annealing	Lower Tm+3	10-30 s	
Forward primer (10 μ M)	3	1	Extension	72	15-40 s/kb	
Reverse primer (10 μ M)	3	1	Final Extension	72	5-10 min	1
Phusion (2 U/ μ L)	0.5	0.2				
Template DNA	X (0.001-250 ng)	X (0.0004-100 ng)				

Phusion DNA Polymerase exhibits 3'→5' exonuclease activity that can degrade primers **in the absence of dNTPs!**

PrimeSTAR HS DNA Polymerase

	50 μ L	20 μ L	Step	Temp, $^{\circ}$ C	Time	Cycles
Water	32.5-X	13.3-X	Initial denaturation	98	30 s-3 min	1
5X PrimeSTAR Buffer	10	4	Denaturation	98	10 s	25-40
dNTP Mix (10 mM each)	1	0.5	Annealing	Tm	5-15 s	
Forward primer (10 μ M)	3	1	Extension	72	1 min/kb	
Reverse primer (10 μ M)	3	1	Final Extension	72	5-10 min	1
PrimeSTAR (2.5 U/ μ L)	0.5	0.2				
Template DNA	X (0.01-200 ng)	X (0.004-100 ng)				

FastDigest Restriction Enzyme

	50 μ L	20 μ L	Step	Temp, $^{\circ}$ C	Time
Water	40-X	16-X	Digestion	37	5-60 min
10X FastDigest Buffer	5	2	Inactivation	65	5 min
DNA	X (< 2 μ g)	X (< 1 μ g)			
FastDigest	5	2			

T4 DNA Ligase

	10 μ L	Step	Temp, $^{\circ}$ C	Time
Water	8.9-X-Y	Ligation	22	10-60 min
Vector DNA	X (10-50 ng)			
Insert DNA	Y (1-5/1 of vector)			
10X Ligase Buffer	1			
T4 DNA Ligase (5 U/ μ L)	0.1			

CPEC

DNA (1:1 Of each piece)
5X HF Phusion Reaction Buffer
10 mM dNTPs
DMSO
2U/μl Phusion Polymerase
dH2O

25ul
x (100ng)
5 μl
1 μl
0.75 μl
0.5 μl
17,75-x

PCR program

30 sec @ 98 C 1 cycle
10 sec @ 98 C }
30 sec @ 55 C } 1 to 15 cycle(s)**
length* (kb) x 15 sec @ 72 C }
10 min @ 72 C 1 cycle
*The total length of the assembled product (in kb)
**The number of repeated cycles should
exceed the number of assembly pieces